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L3: Entry 30 of 34 File: USPT Apr 7, 1992

DOCUMENT-IDENTIFIER: US 5102797 A
TITLE: Introduction of heterologous genes into bacteria using transposon flanked expression cassette and a binary vector system

Detailed Description Paragraph Right (16):
To facilitate insertion of DNA sequences, it is desirable that a carrier plasmid has a minimum number of restriction enzyme sites outside the transposable cassette. The inclusion of the transposase genes on the carrier plasmid would limit its usefulness due to introduction of additional restriction enzyme digestion sites present in the transposase gene sequence. The homologous recombination of the carrier and functions plasmid described in this invention achieves the desirable cis configuration of the transposable element and the transposase genes while allowing for the development of a carrier plasmid with convenient restriction enzyme sites for insertion of DNA sequences.

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L5: Entry 1 of 6 File: USPT Oct 5, 1999

DOCUMENT-IDENTIFIER: US 5961983 A
TITLE: Stable pura vectors and uses therefor

Detailed Description Paragraph Right (57):
A chromosomal integrant of LT-B was obtained in the following manner: A plasmid containing a 31 base inverted repeat derived from the extreme ends of Tn10 and the expressed transposon gene of Tn10 under control of the tac promoter located outside the inverted repeat sequences was used to create a transposon which expressed LT-B. An 800 base pair HaeII restriction enzyme fragment, carrying the lac promoter region and the structural gene for LT-B was cloned into the transposon vector, also containing a kanamycin-resistance determinant. In this configuration, the gene for LT-B and kanamycin resistance were flanked by the inverted repeat sequences. To create a suicide vector for use in Salmonella strains, the transposon carrying LT-B and kanamycin was crossed into a derivative of .lambda.gt11 so that a hybrid phage particle carrying the LT-B transposon and the Tn10 transposase was constructed. A full explanation of this technique can be found in U.S. Ser. No. 07/590,364, filed Sep. 28, 1990, the teachings of which are incorporated herein by reference, and is similar to modified transposons described by Herrero et al., J. Bacteriol. 172: 6537-67 (1990). The modified .lambda. phage carrying the LT-B transposon was used to infect Salmonella LB5010/F112, carrying an expressed .lambda. phage receptor. Since DNA of phage .lambda. does not replicate in Salmonella, clones selected for kanamycin-resistance result from transposition of the modified transposon onto the chromosome. Several independent isolates were obtained; all expressed the LT-B antigen. Several of the S. typhimurium LB5010 isolates were retained and P22 phage lysates propagated on them were used to transduce the chromosomally located expression cassette into the Salmonella vaccine strain SL3261. Two independent SL3261 LT-B chromosomal integrants, resulting from transduction from two separate alleles, located in different loci in the Salmonella chromosome, were found to be stable in vitro and in vivo.

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L4: Entry 10 of 21 File: USPT Jan 26, 1999

DOCUMENT-IDENTIFIER: US 5863726 A
TITLE: Telomerase activity assays

Detailed Description Paragraph Right (11):
In addition to linear single stranded or duplex nucleic acids, the substrate can be a circular plasmid DNA that undergoes linearization at a specific site, either inducibly or spontaneously. Such a plasmid substrate is particularly useful for in situ applications. An illustrative plasmid telomerase substrate is a vector that contains an insert with a unique restriction site (e.g., Isce I) located 3' to the telomerase substrate sequence. In this context, "unique" means that the restriction site is not present in the genome of the cell under analysis. Preferably, the vector is a selectable, multi-copy vector with a mammalian origin of replication. The method can further include a second expression plasmid that contains a gene coding for a restriction enzyme specific for the unique site, under the control of an inducible promoter. The two plasmids are co-infected into the target cell by methods known in the art, and are replicated. Upon induction of the expression plasmid product, the restriction enzyme cleaves the DNA of the telomerase substrate plasmid at the unique restriction site resulting in a linearized substrate plasmid, the ends of which are recognized as a telomerase substrate and can be elongated with TTAGGG repeats by telomerase.

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L4: Entry 2 of 21 File: USPT Aug 7, 2001

DOCUMENT-IDENTIFIER: US 6271205 B1
TITLE: Cancer treatment by expression of differentiation factor receptor

Detailed Description Paragraph Right (38):
To prepare a recombinant retrovirus for expression of TrkA, the vector (for example, pNSV, a Moloney murine leukemia virus vector derived from vector pN2 (Eglitis et al. (1985) Science 230:1395-1398) is cut with a specific restriction enzyme, HindIII, downstream of the SV40 promotor (Kriegler, supra). The part of the trkA cDNA coding for the protein is inserted into the vector and ligated. The ligation mix is then used to transfect Escherichia coli bacteria. Clones are analyzed for a vector with a trkA insert in the correct orientation. The orientation is checked by restriction enzyme digestion with enzymes that cut within the trkA insert. The appropriate clone is grown up and used to transfect a packaging cell line (PA317). The transfection is carried out using DNA precipitated with calcium phosphate (Chang (1994) Calcium Phosphate-Mediated DNA Transfection (Wolff, J. A., ed.), pp. 157-179, Birkhauser, New York). PA317 cells bearing the trkA vector are selected by drug resistance. The vector has a neomycin-resistance nucleic acid, and inclusion of neomycin in the culture medium selects for cells bearing the trkA vector. At this point, the packaging cell line is checked for trkA expression to be certain that the ligation produced the correct virus using immunofluorescence microscopy and a monoclonal antibody specific for TrkA (Ross et al. (1996) J. Cell Biol. 132:945-953. The packaging cell line contains nucleic acids that are required for production of infectious virus. Because the resulting infectious virus is defective, it is competent to infect other cell types but produces infectious virus only from the packaging cell lines. Virus in the culture supernatant is collected and used for therapy. Alternatively, the virus-producing cell line can be grafted in the vicinity of the tumor (Chiocca et al., supra). Using either approach the proliferating tumor cells will become infected and express TrkA. Induction of trkA by any other means is also useful in retarding growth of brain tumors.

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L3: Entry 25 of 34 File: USPT Dec 24, 1996

DOCUMENT-IDENTIFIER: US 5587288 A
TITLE: Regulation of exoprotein in Staphylococcus aureus

Detailed Description Paragraph Right (8):
The transposon Tn917LTV1 inserted into the sar locus of the host chromosome contain an E. coli replicon carrying ampicillin as a selective marker (2). Taking advantage of the unique restriction sites (Xho and Bali) within the transposon, ligation mixture of sar mutant 11D2 (3) chromosomal DNA digest were transformed with one of these enzymes into E. coli strain HB101. Two plasmid clones (pALC1 and pALC2) comprised partly of transposon and adjacent staphylococcal chromosomal sequences were generated. (Table 1). The plasmid pALC1 was purified (13) and digested with XhoI/SaII to release a 4 kb flanking chromosomal fragment which as then cloned into pUC18 to form the pALC3. The 4 kb insert was subsequently released from pUC18 by digestion with SacI/SaII, gel-purified and labeled with .sup.32 P (.alpha.-.sup.32 P deoxycytidine triphosphate, Amersham) (7) to probe a Zap genomic library (Stratagene, La Jolla, Calif.) of S. aureus strain DB as described in the manufacturer's instruction. Two pBluescript phagemids were obtained with inserts of 4.7 and 6 kb, respectively. Plasmids were purified by Magic Maxiprep (Promega, Madison, Wis.). Using both T3 and T7 primers, bidirectional plasmid sequencing was performed with .sup.35 S sequencing mix and Sequenase (US Biochemicals) according to the chain termination method of Sanger (13,19). Additional primers were obtained for sequencing from within the insert. Based on the sequence generated, additional primers were also made to amplify the sar gene by PCR (designated sarA henceforth) from chromosomal DNA of prototypic S. aureus strains RN6390 and RN450 (15). The PCR fragment (732 bp) was cloned into pCRiI (Invitrogen, San Diego, Calif.), cleaved with XbaI/KpnI, and recloned into shuttle vector pSPT1818 (10) in E. coli strain XL-1 blue.

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L4: Entry 5 of 5 File: USPT Sep 3, 1991

DOCUMENT-IDENTIFIER: US 5045461 A
TITLE: Method for increasing yield and nodulation by bradyrhizobium

Detailed Description Paragraph Right (29):
Construction of mutants in nodK. Mutations in the open reading frame designated nodK which precedes the nodABC operon in Bradyrhizobium sp. (Parasponia) strain ANU289 were made by the insertion of a terminatorless kanamycin resistance cassette, derived from Tn5 and cloned in pANU20 (Table 1), into the ClaI site of pPR289-10 located in the nodK gene (FIG. 1). pRPR289-10 is described in Scott, K.F. (1986) supra. Any source of nodK may be used in this invention, however. Any source for the kanamycin resistance gene may also be used with ClaI linkers added as is known to those skilled in the art. The cassette contains a functional notII gene and the first 51 codons of a bleomycin resistance gene immediately 3' to nptII (Mazodier et al. (1985) Nucl. Acids Res. 13:195-205). Kanamycin resistant recombinants were screened by restriction endonuclease analysis and constructs containing the cassette in each orientation were then recloned into the HindIII site of the mobilizable suicide vector pSUP202. This mutated sequence was then homogenotized back into the wild-type strain ANU289 and presumptive mutants screened for double reciprocal crossover as follows. The constructs were transformed into the E. coli strain SM10 and transformants used as donor strains in bacterial crosses with Bradyrhizobium sp. (Parasponia). Streptomycin resistant, kanamycin resistant transconjugants were purified and screened for the loss of pSUP202 sequences by colony hybridization using a labelled pSUP202 DNA probe. Genomic DNA was prepared from those isolates which failed to hybridize to the pSUP202 probe, cleaved with BalII, electrophoresed on an agarose gel, blotted and again probed with pSUP202 sequences, kanamycin resistance cassette sequences and pPR289-10 sequences to verify that a precise double reciprocal crossover in the nodK region had occurred. The pattern of hybridizing bands was consistent with the insertion of the 1.3 kb kanamycin resistance cassette into the ClaI site of nodK. Two isolates corresponding to mutants with the kanamycin resistance cassette in each orientation were identified and designated ANU1291 and ANU3030 respectively (FIG. 1).

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Term: L1 same (degrad$ or suicid$)

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L5	L1 same (degrad$ or suicid$)	6	L5
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L3	L1 same (advantag$ or useful$)	34	L3
L2	L1 same degrad$ same vector$	1	L2
L1	transpos$ same restrict$ same enzyme$	266	L1

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Terms	Documents
L4 same (advantag$ or useful$)	16

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DB=USPT; PLUR=YES; OP=OR			
L5	L4 same (advantag$ or useful$)	16	L5
L4	L1 same degrad$ same (expression near0 vector$)	43	L4
L3	L2 same degrad$ same (expression near0 vector$)	6	L3
L2	L1 same (induc$ or stimulat$ or increas$)	2004	L2
L1	restriction near0 enzyme$	17579	L1

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Terms	Documents
L3 same vector$	21

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DB=USPT; PLUR=YES; OP=OR			
L4	L3 same vector$	21	L4
L3	L1 same (advantag$ or useful$)	34	L3
L2	L1 same degrad$ same vector$	6	L2
L1	induc$ same express$ same (restrict$ near0 enzym$)	772	L1

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Term: T7RNA near0 polymerase near0 promoter$

Display: 10 **Documents in Display Format:** CIT **Starting with Number** 1

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Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT; PLUR=YES; OP=OR			
L8	L4 same (advantag$ or useful$)	0	L8
L7	L4 same link$	1	L7
L6	L4 same induc$	0	L6
L5	L4 same link$ same induc$	0	L5
L4	T7RNA near0 polymerase near0 promoter$	20	L4
L3	T7RNApolymerase near0 promoter$	0	L3
L2	vector$ same (steric near0 hindrance$)	15	L2
L1	vector$ same avoid same (steric near0 hindrance$)	0	L1

END OF SEARCH HISTORY

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L2: Entry 9 of 15 File: USPT Dec 21, 1999

DOCUMENT-IDENTIFIER: US 6004798 A
TITLE: Retroviral envelopes having modified hypervariable polyproline regions

Brief Summary Paragraph Right (6):
Generation of a targeting retroviral vector particle would enable the recombinant retrovirus to deliver a therapeutic gene to the target tissue through cross-species and/or tissue-specific infection. To achieve such a goal, common strategies have been to modify the natural host range determinant, the retroviral envelope protein, by inserting new receptor binding polypeptides into the surface domain (SU) of the envelope protein. The envelope protein, however, is difficult to modify. Prior attempts to modify the retroviral envelope have been directed to the insertion of targeting polypeptides into the receptor binding region of the envelope protein. Such attempts have resulted in the disruption of the envelope structure to such an extent that the folding, processing, and incorporation of the envelope protein is impaired strongly. Also, the activity of the inserted polypeptide may be limited by the steric hindrance caused by the surrounding host protein residues.

Brief Summary Paragraph Right (8):
Applicants have found that the hypervariable polyproline region of the surface domain, which is located next to the receptor binding region, is independent and flexible. The hypervariable polyproline region can be as short as the 11 amino acid residues at the N-terminal and still provide a viral titer of up to 2.times.10.sup.5 cfu/ml. The hypervariable polyproline region also can be made longer than the wild type (i.e., unmodified) hypervariable polyproline region. As shown in Example 6, below, when a collagen binding domain is inserted into the hypervariable polyproline region, the virus which is pseudotyped with such a chimeric envelope can bind to a collagen-coated plate. This indicates that polypeptides inserted into the hypervariable polyproline region can be exposed to the envelope protein surface, and the virus can bind to an extracellular matrix component or a molecule located on the surface of a target cell. Furthermore, the hypervariable polyproline region can be an extended region, which largely eliminates the steric hindrance toward the inserted polypeptide domain caused by the surrounding host protein residues. Thus, applicants have found that one can generate an efficient recombinant retroviral vector particle by inserting a targeting polypeptide into the hypervariable polyproline region of a retroviral envelope protein.

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L4: Entry 15 of 20 File: USPT Sep 8, 1998

DOCUMENT-IDENTIFIER: US 5804190 A
TITLE: Recombinant vaccine for porcine pleuropneumonia

Detailed Description Paragraph Right (13):
Although, appCA genes can be expressed in the recombinant system using the natural A. pleuropneumoniae promoter it is preferable that appCA genes be placed downstream from an appropriate strong promoter and/or amplifier gene. The type of promotor and/or amplifier will depend on the recombinant vector and expression system. Preferred promoters in the current invention are strong bacterial promoters such as the lac or tryp promoters. Examples of other promoters which could be used include the T7RNA polymerase promoter and tac promoter. This will provide for considerably higher levels of expression of antigen. The recombinant vectors containing the DNA sequences described earlier and the recombinant cells containing these DNA sequences which can be utilized to produce A. pleuropneumoniae antigens are covered in this invention.

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